Seasonal Decline in Reproductive Success of the Great Tit

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SEASONAL DECLINE IN REPRODUCTIVE SUCCESS OF THE GREAT TIT: VARIATION IN TIME OR QUALITY?[1]

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Abstract. Seasonal variation in reproductive success is a common feature of most organisms. To understand the evolution of breeding seasons and reproductive strategies of individual animals, it is necessary to assess the extent to which seasonal variation in reproductive success is causally related to seasonal variation in the environment ('timing' hypothesis), to differences in quality between early and late breeders or their territories ('quality' hypothesis), or to a combination of both. We manipulated timing of breeding in the Great Tit *Parus major,* a small passerine, to test these hypotheses. A group of experimentally delayed birds was created by removing first clutches, inducing birds to lay a replacement clutch. Reproductive success of delayed pairs was compared with control pairs that bred early and with pairs that bred late. We conclude that seasonal declines in reproductive success at the nestling stage and survival of adult females were caused by differences in quality between early and late breeders. Recruitment of fledglings into the breeding population and the occurrence of second clutches were causally related to the timing of breeding. The seasonal decline in clutch size was caused by a combination of timing and quality effects. We attempted to assess the relative importance of timing and quality in the seasonal decline in reproductive success, expressed as lifetime production of recruits. We tentatively conclude that 87% of the seasonal decline in lifetime reproductive success could be attributed to a timing effect per se, whereas quality differences between early and late breeders accounted for the remaining 13%.

Key words: clutch size; environmental quality; laying date; Parus major; *phenotypic quality; reproductive success.*

INTRODUCTION

Reproductive success varies seasonally in animal species of diverse taxa, e.g., insects (Ohgushi 1991, Cushman et al. 1994); fish (Schultz 1993); and mammals (Clutton-Brock et al. 1982). Seasonal declines in reproductive success have been particularly well documented in birds (Perrins 1970, Daan et al. 1989). This seasonal decline in reproductive success could be causally related to the timing of breeding, but could also be caused by variation in quality between early and late breeders; i.e., if high-quality birds breed earlier than low-quality birds, this would result in a seasonal decline in reproductive success.

Differences in quality between breeding birds could result from differences in phenotypic quality, e.g., foraging ability, or from differences in environmental quality, e.g., food availability in the territory (see Ens et al. 1992). Similarly, effects of timing of breeding can be subdivided into separate categories. Absolute timing (calendar date) may be important when, for example, birds have to molt or migrate before winter. Timing relative to phenology of other organisms may be important when breeding is synchronized with seasonal fluctuations in the population of prey species (van Balen 1973). Timing relative to conspecifics may be important when age or prior occupancy play a role in territory settlement (Nilsson 1990, Sandell and Smith 1991), or, for colonial breeders, when synchronization with other members of the colony reduces predation rate (Parsons 1975, Hatchwell 1991).

To understand phenotypic variation in breeding dates in an evolutionary context requires knowledge of the fitness consequences of alternative breeding dates. In other words, it requires that we establish if there is a causal relationship between timing of reproduction and reproductive success (Daan et al. 1990, Hochachka 1990). To disentangle the effects of quality and the timing of breeding on reproductive success, experi-

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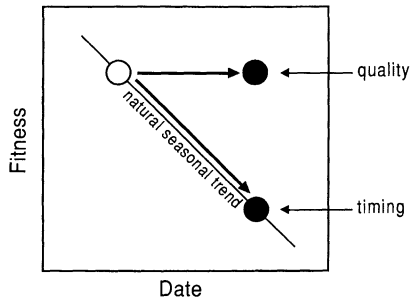

FIG. 1. Predicted experimental results for the 'timing' and 'quality' hypotheses in Great Tits. Control birds, ○; experimentally delayed birds, ●.

ments that manipulate the timing of breeding are needed. Such studies are relatively rare (Parsons 1975, Hunt and Hunt 1976, Dijkstra 1988, Nilsson 1990, Hatchwell 1991, Norris 1993, Brinkhof et al. 1993, Wiggins et al. 1994), and have focused exclusively on fitness components of the manipulated clutch, while ignoring possible effects of the timing of breeding on future reproductive output of the parents. However, selection acts on inclusive fitness, which includes both current and future clutches, so that evaluation of the effect of a trait on fitness requires accounting for effects on parents as well as offspring.

In this paper, we report results from an experiment testing the hypothesis that there is a causal relationship between reproductive success and timing of breeding in Great Tits, *Parus major*. We manipulated timing of breeding during four years by removing first clutches to induce birds to lay replacement clutches, thereby creating a group of experimentally delayed breeding pairs. The 'timing' and 'quality' hypotheses yield unique predictions, which are schematically illustrated in Fig. 1. The comparison between control pairs and experimentally delayed pairs yields information on the effect of timing; i.e., if experimental birds have lower reproductive success than control birds, then there is evidence of a causal relationship between timing and success of reproduction. The comparison between experimentally delayed pairs and the natural seasonal trend yields information on the effect of quality. If experimental birds have higher reproductive success than control birds breeding at the same time, then we would conclude that there is a difference in quality between early and late breeders, which contributes to the seasonal decline in reproductive success. This natural seasonal trend in reproductive success is calculated using all unmanipulated clutches. Intermediate results are also possible because the two hypotheses are not mutually exclusive.

Timing of breeding cannot be manipulated without causing some form of experimental bias. In our study, delayed females had to lay an extra clutch, which could have affected their quality, e.g., their nutrient reserves. Although we acknowledge that such biases may exist, we found no evidence of negative effects associated with laying a replacement clutch (see *Discussion*).

Preliminary conclusions, based on the first year of this study only, have already been published (Verhulst and Tinbergen 1991). In that paper, we concluded that the seasonal decline in pre-fledging reproductive success was caused by differences in quality between early and late breeders, whereas the seasonal decline in post-fledging reproductive success was causally related to timing of breeding. In this paper, we analyze the effects of the experiment on pre-fledging reproductive success, post-fledging reproductive success, and future reproductive output of the parents during all four years of the study. We then assess the relative importance of timing and quality in the seasonal decline in reproductive success, by integrating the experimental effects on different fitness components into one measure of fitness (lifetime reproductive success).

METHODS

Field work was conducted in Oosterhout, an 11.4-ha deciduous forest located in the Netherlands, dominated by mature oaks, *Quercus robur*. A box-nesting population of Great Tits has been studied in this forest since 1955, and the study area and general methods are described in detail by van Balen (1973). During the breeding season, all nest boxes were checked at least once a week. The following variables were recorded: clutch size, hatching date, number of eggs hatched, number of young fledged, and body mass of young when 14 d old (day of hatching = 0). Laying date of the first egg was calculated, assuming that 1 egg/d was laid during the laying period. Hatching date was established by inspecting the nest box around the expected day of hatching. The number of young fledged was established by searching the nest for dead banded nestlings after fledging. Parents were captured in the nest box when the young were ≈7 d old. All young and parents caught were banded, and their local survival was estimated by catching breeding birds in the following year. Thus, local survival is defined as the probability of recapture as a breeding bird in the study area in the next year. Since most breeding adults were captured in all years, and capture probability was independent of timing of breeding in the experimental years (J. H. van Balen, *unpublished data*), we did not correct for capture effort. Great Tits usually start breeding when they are 1 yr old and generally breed in each year of their life.

Dates are presented as "aprildate" (1 April = day 1; 1 May = day 31, etc.).

Experimental protocol

Clutches were checked daily when they were nearly complete. Although cold periods sometimes cause interruptions of egg laying (Kluyver 1951), we assumed clutches to be complete on the first day that no additional egg was laid. We selected pairs of completed

clutches with the same laying date and clutch size (to within 1 d and egg). In each pair, one randomly chosen clutch was removed while the other served as a control. In the first year (1987), there were often two control clutches for each removed clutch. The clutches were removed as soon as possible after the last egg was laid.

In 1987, 1989, and 1990, eggs of removed and replacement clutches were measured for length and width to the nearest 0.1 mm. Egg volume was calculated using the equation developed by van Noordwijk et al. (1981*a*).

In 1987, before clutch removal, incubating females were individually marked with dyes on the white vanes of the outer tail feathers to facilitate recognition of replacement clutches. In later years, incubating experimental females were taken from the nest before clutch removal and identified with metal or color bands. All females found incubating were checked for paint marks (in 1987) or color bands (in later years), and they were assumed to retain the same mate while rearing the replacement clutch. Replacement clutches were usually laid in a nest box close to the nest box where the first clutch was laid, and occasionally in the same nest box.

Data analysis

A descriptive analysis of the natural seasonal trends in clutch size and components of reproductive success was carried out, and the contribution of the clutch to fitness was examined in four phases: clutch size, probability of success (fledging at least one young), the number of fledglings per egg in successful nests, and recruitment of fledglings into the breeding population. With respect to the future reproductive success of the parent, we investigated the effect of the experiment on the occurrence of second clutches and survival until the next breeding season. All genuine first clutches laid in the years 1977 to 1991 that had not been involved in experiments were used in this analysis.

Variables included in the analysis were: year (as a factor or categorical variable), date (laying date of the first egg in the analysis of seasonal variation in clutch size, and hatching date in all other analyses), date squared, and the interaction between year and date. When date squared contributed significantly to the explained variance, the interaction term between year and date squared was also tested.

Data were analyzed with the GLIM statistical package (Numerical Algorithms Group 1987) with normal or binomial error distribution, using a stepwise backward procedure. Binomial error (or logistic regression, Sokal and Rohlf 1994) was used when analyzing variation in proportions, such as fledging success or survival rate. Significance was tested with the chi-square test when the scale parameter (deviance/df) was <1, and with the F test when the scale parameter was >1. Thus P values are conservative. All statistical tests are two-tailed.

TABLE 1. Laying date and clutch size of control, removed, and experimental clutches of the Great Tit in Oosterhout, The Netherlands. For laying date, 1 April = day 1. *N*, no. of clutches.

Year	Laying date		Clutch size		
	$\bar{X}$	SD	$\bar{X}$	SD	*N*
1987*					
Control	22.1	2.4	10.0	1.1	23
Removed	22.1	2.4	9.6	1.1	13
Experimental	37.9	2.7	9.4†	0.8	10
1989					
Control	12.3	4.5	9.1	1.4	16
Removed	11.9	4.5	9.7	1.2	16
Experimental	30.2	3.6	8.9†	0.8	14
1990					
Control	7.5	3.7	9.2	1.3	20
Removed	7.3	4.0	8.7	1.2	20
Experimental	23.9	3.4	8.7†	1.0	17
1991					
Control	10.5	4.1	9.4	1.5	17
Removed	10.6	3.6	9.3	1.2	17
Experimental	28.8	5.2	8.0†	1.2	14

* Data from Verhulst and Tinbergen (1991).

† Clutch size of experimental clutches was significantly smaller than for removed clutches in 1991 (paired t test, difference -1.4 egg, $t_{13} = 3.82$, $P < 0.003$), but not in any other year (1987: difference -0.3 egg, $t_9 = 0.63$, $P > 0.2$; 1989: difference -0.6 egg, $t_{13} = 1.56$, $P = 0.07$; 1990: difference -0.1 egg, $t_{16} = 0.52$, $P > 0.3$). Note that only those removed clutches that resulted in an experimental clutch were used in pairwise tests.

RESULTS

Laying date and clutch size of control and removed clutches were not significantly different in any year (Table 1). Clutch removal took place 2.0 ± 1.7 d (mean ± 1 SD; $N = 66$) after the last egg of the clutch had been laid. Replacement clutches were initiated following 83% (55/66) of the clutch removals, and this proportion did not vary among years ($\chi^2 = 0.62$, df = 3, $P > 0.8$). Because the color dyes used in 1987 tended to fade, and because (in all years) birds could have started replacement clutches in a natural cavity and therefore gone undetected, we probably underestimated the true proportion of birds renesting. The probability that clutch removal resulted in a replacement clutch was independent of laying date, clutch size, and removal date (logistic regression, $P > 0.5$ for all variables). We therefore assumed that the birds starting replacement clutches were an unbiased sample of the group from which clutches were removed.

The average time between clutch removal and initiation of the replacement clutches was 5.7, 6.1, 6.9, and 7.7 d during 1987, 1989, 1990, and 1991, respectively (range 4–12 d). This interval decreased as the date of clutch removal became later in the season ($R^2 = 0.16$, $N = 55$, $P < 0.003$), but was independent of laying date ($F_{1,52} = 0.3$, $P > 0.5$), clutch size ($F_{1,52} = 0.7$, $P > 0.7$), and year ($F_{3,50} = 2.5$, $P = 0.07$), when removal date was controlled for. Replacement clutches

TABLE 2. Results of the analysis of natural seasonal trends in clutch size, reproductive success of first clutches in different reproductive stages, and female survival in Great Tits, Oosterhout, The Netherlands.

Reproductive stage	Parameter*	Change in: Deviance	Change in: df	P†
Clutch size	Null model	1533.7	579	
	Final model	1270.8	563	
	Constant		+1	
	Year	+236.2	+14	<0.0001
	Laying date	+39.7	+1	<0.0001
	Square laying date	+14.4	+1	<0.02
Fledglings per egg	Null model	955.8	431	
	Final model	814.1	400	
	Constant		+1	
	Year	+67.0	+14	<0.004
	Hatching date		+1	
	Year × hatching date	+61.4	+14	<0.01
	Square hatching date		+1	
	Period × square hatching date	+19.4	+1	<0.003
Local survival fledglings	Null model	605.2	431	
	Final model	480.7	401	
	Constant		+1	
	Year	+49.6	+14	<0.001
	Hatching date		+1	
	Year × hatching date	+49.9	+14	<0.001
	Square hatching date	+4.7	+1	<0.05
Local survival females	Null model	591.3	426	
	Final model	566.0	411	
	Constant		+1	
	Year	+22.7	+14	>0.2
	Hatching date of young	+10.6	+1	<0.006

* The null model includes the constant only; the final model includes all shown parameters. Sample size is equal to the number of df in the null model + 1.

† Significance was tested by dropping parameters one at a time from the final model, using the change in deviance in combination with change in degrees of freedom.

were initiated, on average, 15.8, 18.3, 16.6, and 18.2 d after initiation of the removed clutch in 1987, 1989, 1990, and 1991, respectively. Replacement clutches will henceforth be referred to as the experimental clutches.

For clutch size and different fitness components, we (1) evaluate the natural seasonal trend among unmanipulated clutches, (2) compare experimentally delayed and control pairs to test for a causal relationship between reproductive success and timing of breeding, and (3) compare experimentally delayed pairs with pairs that naturally breed late to determine whether or not quality differences between early and late breeders play a role in the natural seasonal trend in reproductive success.

Clutch size and egg volume

The natural seasonal trend in clutch size was analyzed using multiple regression. Year (as a categorical variable), laying date, and laying date squared explained a significant part of the variance (Table 2), and the decline in clutch size within seasons was concave up (Fig. 2A). There was no significant interaction between year and laying date ($F_{14, 549} = 1.1$, $P > 0.3$) or between year and laying date squared ($F_{14, 535} = 1.1$, $P > 0.3$). The quadratic term (Table 2) suggests that clutch size increases when birds start laying after day 35. However, estimates of clutch size after day 35 were largely extrapolations, as <2% of all first clutches were initiated after this date.

In 1991, clutch size was significantly smaller in experimental than in removed clutches, but not in the other three years (Table 1). When experimental years were pooled and only removed and experimental clutches were included in the analysis, there was no significant interaction between year and manipulation category ($F_{3, 112} = 2.0$, $P > 0.1$). However, controlling for year ($F_{3, 115} = 3.8$, $P < 0.02$), experimental clutches had, on average, 0.57 fewer eggs than removed clutches ($F_{1, 115} = 6.8$, $P = 0.01$). We therefore conclude that at least part of the seasonal decline in clutch size was causally related to timing of laying.

We investigated whether or not clutch size in experimental clutches deviated from values expected on the basis of the natural seasonal trend. This test was carried out by adding data from the experimental clutches to data on unmanipulated clutches (Table 2) and including clutch type (unmanipulated or experimental) as a factor in the model presented in Table 2. Experimental data were added for each year separately and for all years combined. Unavoidably, our estimate of the natural seasonal trend over the period when the

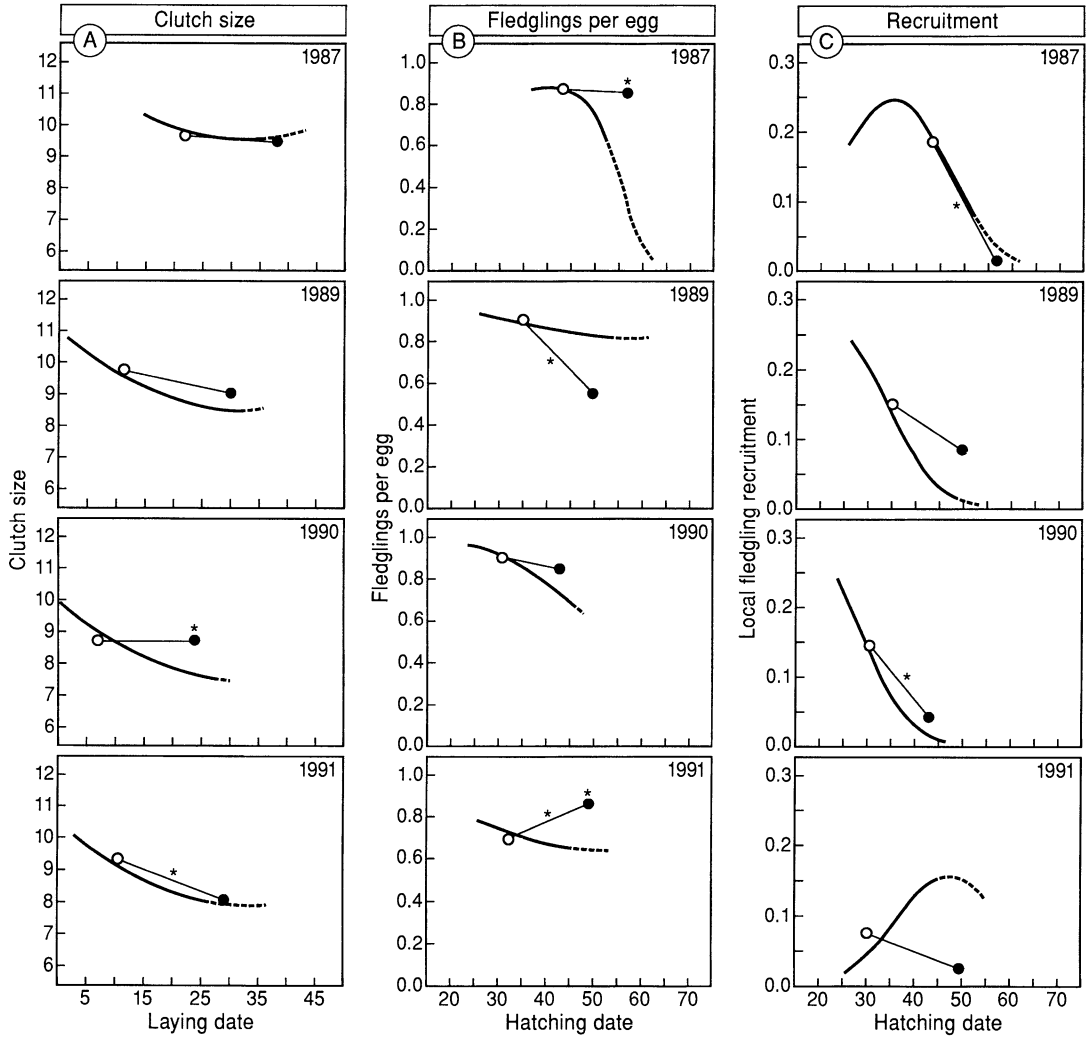

FIG. 2. Experimental results and natural seasonal trend in 1987 and 1989–1991 in Great Tit (A) clutch size, (B) no. of fledglings per egg, and (C) local recruitment of fledged young. Laying date: 1 April = day 1. Data from 1987 taken from Verhulst and Tinbergen 1991. Control clutches, ○; experimentally delayed clutches, ●. A significant difference between experimental and control clutches is indicated with an asterisk on the line connecting the data points. A significant difference between the experimental data and the natural seasonal trend is indicated with an asterisk near the experimental data point; statistics: (A) 1987: $F_{1, 572} = 0.0$, $P > 0.9$; 1989: $F_{1, 576} = 1.3$, $P > 0.2$; 1990: $F_{1, 579} = 7.5$, $P < 0.01$; 1991: $F_{1, 576} = 0.1$, $P > 0.7$; (B) 1987: $F_{1, 408} = 5.47$, $P < 0.02$; 1989: $F_{1, 412} = 2.29$, $P > 0.1$; 1990: $F_{1, 412} = 1.18$, $P > 0.2$; 1991: $F_{1, 412} = 7.67$, $P < 0.006$; (C) 1987: $F_{1, 409} = 1.7$, $P > 0.2$; 1989: $F_{1, 413} = 1.0$, $P > 0.3$; 1990: $F_{1, 413} = 0.04$, $P > 0.8$; 1991: $F_{1, 414} = 2.95$, $P > 0.8$. See Table 3 for additional statistical details.

experimental clutches were laid was, to some extent, an extrapolation in every year (Fig. 2). In 1990, experimental clutches were significantly larger than expected on the basis of the natural seasonal trend (Fig. 2A). In the other three years, size of experimental clutches did not differ significantly from values expected on the basis of the natural seasonal trend. However, when years were pooled, experimental clutches averaged 0.57 egg more than expected on the basis of the natural seasonal trend, and this effect was significant ($F_{1, 617} = 3.9$, $P < 0.05$). We therefore conclude that, in addition to the date effect described above, seasonal variation in clutch size was also caused by differences in quality between early and late breeders.

Eggs in experimental clutches were consistently larger than eggs in the removed clutch during all three years in which eggs of both removed and experimental clutches were measured (paired t test; 1987: difference = 0.135 cm^3, $P < 0.0001$; 1989: difference = 0.061 cm^3, $P < 0.01$; 1990: difference = 0.081 cm^3, $P < 0.001$).

Fledglings per egg and nestling mass

Among unmanipulated first clutches in the years 1977–1991, the probability was 0.89 ($N = 488$ clutch-

TABLE 3. Experimental results for the Great Tit. Mean value ($\bar{X}$), standard deviation (SD), and sample size (N) are given per year for control and experimental clutches. Clutches that failed completely (no young fledged) are excluded from the calculation of number of fledglings per egg and female survival. All P values refer to a comparison between control and experimental clutches within years, using logistic regression for fledglings per egg and fledgling recruitment, Student's t test for nestling weight, and chi-square for all other tests.

		Control			Experimental			
	Year	$\bar{X}$	SD	N	$\bar{X}$	SD	N	P
Hatching date	1987*	43.1	2.8	23	56.6	3.1	10	
	1989	35.1	4.2	16	49.5	3.6	14	
	1990	30.8	3.9	20	42.9	3.2	16	
	1991	30.5	8.8	17	49.5	4.1	13	
Fledglings per egg	1987*	0.86	0.14	23	0.85	0.23	9	>0.6
	1989	0.89	0.12	16	0.54	0.30	13	<0.0001
	1990	0.89	0.13	19	0.84	0.16	14	>0.3
	1991	0.67	0.23	15	0.89	0.10	13	<0.0003
Nestling weight	1987†	17.6	0.7	23	17.4	0.7	8	>0.5
	1989	17.2	0.8	15	16.5	1.8	13	>0.2
	1990	17.6	0.9	18	16.0	1.3	14	<0.0003
	1991	16.3	1.6	12	17.6	0.9	10	<0.04
Local survival fledglings	1987†	0.16		23	0.01		9	<0.005
	1989	0.14		16	0.08		13	>0.3
	1990	0.14		19	0.04		14	<0.03
	1991	0.08		15	0.02		13	>0.2
Local survival females	1987*	0.61		23	0.22		9	<0.05
	1989	0.38		16	0.69		13	>0.08
	1990	0.33		18	0.43		14	>0.5
	1991	0.33		15	0.46		13	>0.4
Local survival males	1987*	0.45		20	0.50		6	>0.8
	1989	0.60		15	0.36		14	>0.1
	1990	0.18		17	0.92		13	<0.001
	1991	0.20		15	0.33		12	>0.4

* Data from Verhulst and Tinbergen (1991).
† Adapted from Verhulst and Tinbergen (1991).

es) that a clutch was successful (at least one young fledged). The effects of year and laying date on the probability of clutch success were analyzed using logistic regression. When we controlled for year ($\chi^2 = 30.3$, df = 14, $P < 0.01$) the probability that a clutch was successful was unrelated to laying date ($\chi^2 = 0.01$, df = 1, $P > 0.9$). In agreement with the natural seasonal trend, the probability of success did not differ between control and experimental clutches in any year, or in a combined analysis of all four years (control: 73/76 clutches successful; experimental: 49/55 clutches successful; $\chi^2 = 2.4$, df = 1, $P > 0.1$).

Failed clutches were excluded from analysis of the relationship between date and the proportion of eggs resulting in fledged young (= fledging success) because total failure can probably be attributed to factors other than those causing variation in fledging success among successful nests. Using logistic regression, we constructed a model containing year, hatching date (interacting with year), and hatching date squared. Each brood was treated as one degree of freedom in this analysis. In contrast to our earlier report (Verhulst and Tinbergen 1991), hatching date squared was not significant in this analysis. The interaction between year and hatching date squared was not significant ($F_{14, 386} = 1.2$, $P > 0.2$). To investigate whether or not there had been a change in the natural seasonal trend in fledging success since our last investigation, we tested for a significant interaction between hatching date squared and time period (the last three years (1989–1991) of our study vs. the 12 years (1977–1988) used in our previous analysis). This interaction explained a significant part of the variance (Table 2), indicating that a change in the natural seasonal trend in fledging success had occurred. This change coincided with a decrease in caterpillar abundance that will be discussed elsewhere (J. M. Tinbergen et al., *unpublished data*). In summary, eggs from late clutches had a reduced probability of producing fledglings as compared to eggs in early clutches, but the shape of this relationship differed between years (Fig. 2B).

Experimental effects on fledging success varied markedly among years (Table 3, Fig. 2B). In 1987 and 1990, there was no difference between control and experimental clutches; in 1989, fledging success in experimental clutches was significantly reduced; in 1991, fledging success was significantly higher in experimental clutches. When years were pooled, there was no net experimental effect ($F_{1, 117} = 1.9$, $P > 0.15$, controlling for year). We therefore conclude that there was no consistent causal relationship between fledging success and hatching date.

Fledging success in experimental clutches was compared with values expected on the basis of the natural seasonal trend. This was carried out by adding the experimental data to the data set used in the descriptive analysis of the natural seasonal trend (Table 2). This was first done for each year separately and then for all years combined. In two out of four years, fledging success was higher than expected on the basis of the natural seasonal trend, but there was no significant difference in the remaining two years (Fig. 2B). When the experimental data were pooled, fledging success in experimental clutches was higher than expected on the basis of the natural seasonal trend ($F_{1, 446} = 3.8$, $P = 0.05$). We therefore conclude that seasonal variation in fledging success was caused by differences in quality between early and late breeders, although the results suggest that the importance of this quality effect varies between years.

Nestling mass has a strong effect on local recruitment rate (Tinbergen and Boerlijst 1990) and is therefore an indication of the contribution to fitness of the brood. We did not analyze the natural seasonal trend, but nestling mass varied largely in parallel with the number of fledglings per egg (Table 3). Compared with control clutches, nestlings in experimental clutches had lower mass in one year, higher mass in another year, and no significant difference in the two remaining years (Table 3). When years were pooled and controlled for ($F_{3, 108} = 1.4$, $P > 0.2$), nestling mass tended to be lower in experimental clutches ($F_{1, 108} = 3.6$, $P = 0.06$; difference 0.45 g).

Fledgling recruitment

The natural seasonal trend in local recruitment rate of fledglings was analyzed using logistic regression. Each brood was treated as a separate unit in this analysis, rather than each fledgling as in our previous analysis (Verhulst and Tinbergen 1991). Year, hatching date (interacting with year), and hatching date squared explained a significant part of the variance (Table 2). The interaction between year and hatching date squared did not significantly contribute to the explained variance ($F_{14, 387} = 1.1$, $P > 0.3$). Local recruitment of late fledglings was lower than recruitment of early fledglings in most years. However, the slope varied between years (Fig. 2C) and was positive in 4 out of 15 years, including one of the experimental years (1991).

Fledgling recruitment was significantly lower from experimental than from control clutches in two out of four years (Table 3). When years were pooled, the overall difference in recruitment rate between fledglings from control and experimental clutches was significant ($F_{1, 120} = 17.5$, $P < 0.0001$), and there were no significant differences in slope ($F_{3, 114} = 0.9$, $P > 0.4$) or elevation ($F_{3, 117} = 1.1$, $P > 0.3$) among years. We therefore conclude that there was a causal relationship between timing of breeding and fledgling recruitment.

Fledgling recruitment from experimental clutches did not differ in any year from values expected on the basis of the natural seasonal trend (Fig. 2C), or when the experimental data were pooled ($F_{1, 448} = 0.5$, $P > 0.4$). Thus there was no indication that potential quality differences between early and late breeders played a role in the seasonal decline in fledgling recruitment.

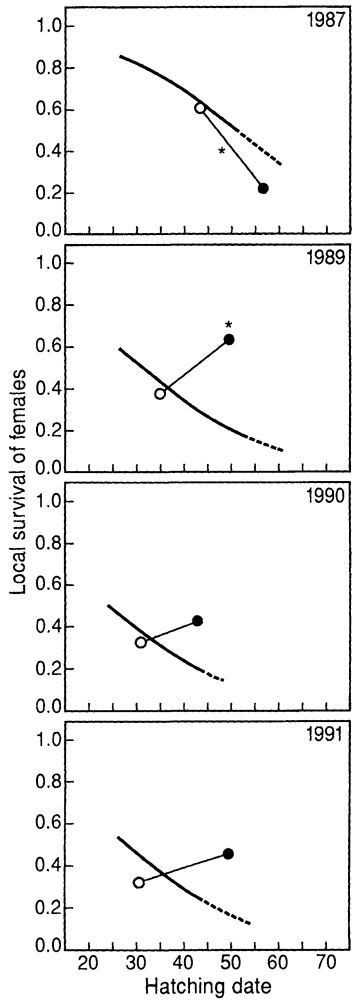

FIG. 3. Experimental results and natural seasonal trend in local survival of female breeding Great Tits. Symbols as in Fig. 3. Hatching date: 1 April–day 1. Data from 1987 taken from Verhulst and Tinbergen (1991). Statistics (see text for methods): 1987: $F_{1, 438} = 0.73$, $P > 0.3$; 1989: $F_{1, 443} = 5.17$, $P < 0.03$; 1990: $F_{1, 445} = 1.09$, $P > 0.2$; 1991: $F_{1, 442} = 2.84$, $P > 0.09$.

Future reproductive success of the parents: second clutches

Great Tits are facultatively multibrooded. Second clutches are defined as clutches that are initiated after successfully rearing another clutch in the same season. A variable proportion of pairs lays a second clutch, and in some years, some birds will even lay a third clutch after having raised young from two previous broods (Tinbergen and van Balen 1988). However, the fre-

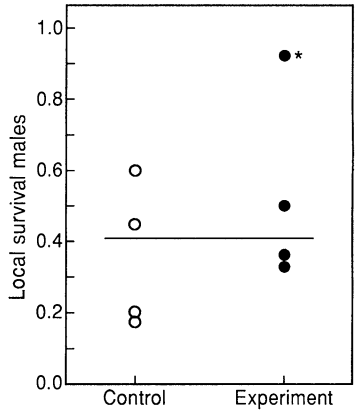

FIG. 4. Experimental results and long-term average value (solid line) of male Great Tit survival. Control (○) and experimental (●) clutches. Point marked with asterisk differed significantly from control group within that year. See Table 3 for statistical details. Data from 1987 taken from Verhulst and Tinbergen (1991).

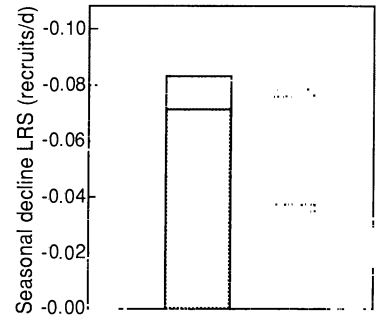

FIG. 5. The slope of the seasonal decline in lifetime reproductive success (LRS) of Great Tits (in recruits per day). Indicated are the contributions of the timing effect and the quality effect.

quency of second clutches in Oosterhout was low, as is the rule in areas with a high density of breeding pairs (Kluyver 1951).

In the experimental years, there was a seasonal decline in the probability that a pair would lay a second clutch, and the level of this relationship differed among years (logistic regression: $N = 73$, laying date: $\chi^2 = 12.4$, df = 1, $P < 0.001$; year: $\chi^2 = 11.0$, df = 3, $P < 0.02$).

When years were combined, significantly more control pairs than experimental pairs started a second clutch, indicating that the seasonal decline in initiation of second clutches was causally related to timing of breeding (control pairs: 8/73; experimental pairs: 0/49; $\chi^2 = 5.7$, df = 1, $P < 0.02$). No experimental pair started a second clutch, in agreement with the natural seasonal trend (tested by adding manipulation category to the regression model containing laying date and year: $\chi^2 = 0.0$, df = 1, $P > 0.9$).

Adult survival

The natural seasonal trend in adult female survival was investigated using all successful first clutches (at least one young fledged) that had not been involved in experiments. There was a seasonal decline in local survival of breeding females when we controlled for year (Table 2); the slope of this decline did not vary significantly among years ($F_{14, 397} = 0.9$, $P > 0.5$).

The effect of the experiment on local survival of adult females was investigated for successful pairs. Local survival of experimental females was significantly lower than that of control females in 1987, but no significant difference was found in any other year (Table 3). When years were pooled, there was no significant effect of the experiment on female survival ($\chi^2 = 0.2$, df = 1, $P > 0.6$). Thus, there was no evidence for a causal relationship between timing of breeding and female survival.

Local survival of females was significantly higher than expected on the basis of the natural seasonal trend in 1989, but not in any other year (Fig. 3). When the experimental data were pooled, experimentally delayed females survived significantly better than expected on the basis of the natural seasonal trend ($F_{1, 459} = 4.5$, $P < 0.05$). We therefore conclude that the seasonal decline in female survival is caused by differences in quality between early and late breeders.

There was no significant seasonal variation in survival of male breeders and no significant variation among years. Local survival did not differ systematically between control and experimental males, although survival of experimental males was significantly higher than survival of control males in 1990 (Table 3, Fig. 4). During 1990 survival of experimental males was unusually high (12/13), and we have no explanation for this result. Due to 1990 data, there was a nonsignificant tendency for experimental males to survive better than control males when years were pooled ($\chi^2 = 3.37$, df = 1, $P = 0.07$). Since the effect was not significant when years were pooled, and we believe that 1990 results represent a statistical artifact, we conclude that local survival of breeding males is independent of timing of breeding.

Integration of parent and offspring fitness

To assess the relative contributions of the effects of timing and quality, we estimated the effect of timing of breeding on fitness, including both the first clutch and the future reproductive output of the parents. Because females lay the eggs, calculations were based on the female's perspective. As the fitness currency, we used the expected lifetime production of recruits. The calculation and some assumptions are summarized in the Appendix; the main result is shown in Fig. 5. We conclude that the seasonal decline in reproductive success was largely (≈87%) causally related to timing of breeding while the remaining part of the seasonal de-

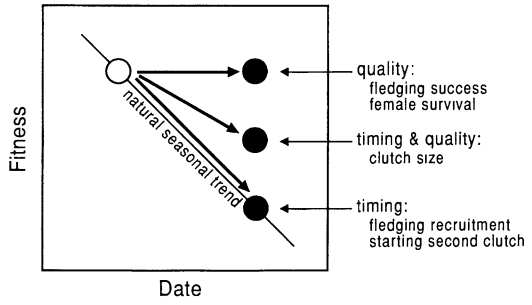

FIG. 6. Schematic summary of the conclusions regarding contributions of quality and timing to seasonal decline in reproductive success. Control birds; ○; experimentally delayed birds, ●

cline (≈13%) was caused by quality differences between early and late breeders.

DISCUSSION

An experimental bias?

Timing of breeding cannot be manipulated without causing some form of experimental bias. In our study, the experimental pairs were forced to lay another clutch and this may have affected their phenotypic quality. We therefore cannot exclude the possibility that a reduction in reproductive success was caused by an experimental bias rather than by seasonal variation in the environment. However, females succeeded in laying larger eggs in the experimental clutch. In the Great Tit, larger eggs contain more energy (Ojanen 1983). Furthermore, fledging success and adult survival of experimental birds were unaffected. We therefore assume that the experimental effects were not primarily due to an adverse effect on parental quality.

Effects of timing and quality on clutch size and reproductive success

Our conclusions are schematically summarized in Fig. 6. We concluded that seasonal declines in fledging success and adult female survival were caused by differences in quality between early and late breeders, whereas the seasonal declines in fledgling recruitment and the occurrence of second clutches were causally related to timing of breeding. With respect to female survival, this conclusion contrasts with our earlier report based only on 1987 data (Verhulst and Tinbergen 1991), in which we concluded that the seasonal decline in female survival was causally related to time of breeding. The seasonal decline in clutch size was caused by a combination of timing and quality effects. Adult male survival and the probability of clutch success were independent of timing of breeding.

Although our results suggest that seasonal variation in fledging success was due to quality effects only, there is evidence to suggest that seasonally declining fledging success was caused by a combination of timing and quality effects. The results were strongly influenced by data collected in 1991, which was an exceptional season (see also Nilsson 1994). In this year, fledging success of experimental pairs was significantly higher than fledging success of control pairs (Table 3).

The contributions to fitness made by the clutch and future reproductive output were integrated into one measure: expected lifetime production of recruits. We concluded that 87% of the seasonal decline in reproductive success could be causally related to timing of breeding. The remaining 13% was due to quality differences between early- and late-breeding birds. Although the precision of these estimates is probably not very high, we think it is safe to conclude that the timing effect was much more important than the quality effect. The overriding effect of timing of breeding was due to the seasonal decline in fledgling recruitment, which was causally related to date.

The conclusion that the seasonal decline in reproductive success was largely due to a timing effect does not imply that differences in quality are unimportant as a source of variation in reproductive success. Quality may determine the timing of laying, with high quality pairs (or pairs with high quality territories) laying earlier than low quality pairs. For example, Gustafsson et al. (1994) showed, with serological data, that early-breeding Collared Flycatchers *Ficedula albicollis* were in better health than late-breeding birds. Secondly, quality may play a role independent of the timing of breeding, as in the European Coot *Fulica atra* (Brinkhof et al. 1993).

Possible mechanisms

The seasonal decline in clutch size was attributed to a combination of timing and quality effects. This implies that the seasonal decline in clutch size, as found at the population level, is also present in individual birds; however, part of the seasonal decline is caused by females that lay smaller clutches (independent of timing of breeding) late in the season. A similar pattern has been observed in Song Sparrows *Melospiza melodia* (Hochachka 1990). Haywood and Perrins (1992) found a correlation between nestling mass of female Great Tits and the size of clutches they laid later in life, which suggests that part of the variation in quality between birds may be caused by variation in the quality of the environment during early development.

The seasonal declines in fledging success and female survival were attributed to variation in quality between early and late breeders. Differences in environmental quality, as well as differences in phenotypic quality, could have caused these effects, but there are no data available to discriminate between these possibilities. In the Great Tit, as in many other species, older females lay earlier (reviewed by Perdeck and Cavé 1992). Although this could be interpreted as an indication that early and late females differ in phenotypic quality, environmental quality could also vary systematically with

age, provided females live in better territories as they get older.

The fitness component that contributed most to the seasonal decline in fitness of the clutch was local recruitment of fledglings. This was the only fitness component of the clutch where we found no evidence that quality differences between early and late breeders played a role. Differences in mass between early and late fledglings may have played a role, because fledgling mass and recruitment rate are correlated (Perrins 1965, Tinbergen and Boerlijst 1990, Lindén et al. 1992). However, the seasonal decline in local survival remains significant when nestling mass is taken into account (Lindén et al. 1992; S. Verhulst, *unpublished data*), indicating that time of birth affects fledgling recruitment independent of fledgling mass. This is illustrated by the fact that, in our study, local survival of fledglings from experimental clutches was lower in all four years, regardless of whether nestling mass in experimental clutches was higher, lower, or equal to nestling mass in control clutches (Table 3).

Since we estimated local survival rather than total survival, seasonal variation in dispersal rate may have contributed to the disappearance of late fledglings. Dhondt and Hublé (1968) suggested that there was no difference in survival between early- and late-hatched birds when dispersal was taken into account. However, their analysis pooled years; this may have affected their results because late fledglings (from second clutches) are relatively more numerous in 'good' years, characterized by low breeding density and a large beech crop, both of which enhance survival of late-hatched fledglings (van Balen 1980, Tinbergen et al. 1985). In a study of Great Tits breeding in Wytham Wood (near Oxford, UK), which is comparable to our study area with respect to vegetation and breeding density, hatching date did not affect the probability of dispersal into surrounding woodlands and hedgerows when year was controlled for (S. Verhulst, C. M. Perrins, and R. Riddington, *unpublished data*). This suggests that the seasonal decline in fledgling recruitment cannot be attributed to a seasonal change in dispersal rate.

Competition between early and late fledglings may have played a role in the disappearance of late fledglings (Kluyver 1971), because age and prior occupancy are important predictors of dominance (Drent 1983, Sandell and Smith 1991). Nilsson (1990) released juvenile Marsh Tits *Parus palustris* in the field at different dates and found that early-released birds were more likely to establish themselves in winter flocks (a prerequisite to establishment as a breeding bird). Further confirmation of our hypothesis comes from experiments with Great Tits in which density of early fledglings was reduced, thereby enhancing local survival of late fledglings (Tinbergen et al. 1985, Verhulst 1992). If competition between early and late fledglings caused the seasonal decline in fledgling recruitment, this implies that the optimal time of breeding depends on the timing of breeding of the other birds in the population.

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APPENDIX

Expected lifetime reproductive success was calculated for early breeders (the control group), delayed breeders (the experimental group), and spontaneously late breeders (the late group, breeding at the same time as the experimentally delayed birds). First, the average number of recruits per breeding season (NR_{yr}) was calculated as follows:

$$NR_{yr} = P_{suc}\cdot[CS\cdot FE\cdot FR + P_2\cdot NR_2] + [1 - P_{suc}]\cdot P_R\cdot NR_R.$$

P_{suc} is the probability that at least one young fledged, CS is clutch size, FE is the proportion of eggs resulting in a fledged young in successful clutches, FR is the probability that a fledged young is recaptured as breeding bird (=recruit), P_2 is the probability that a pair starts a second clutch after successfully fledging young from the first clutch, NR_2 is the average number of recruits per second clutch, P_R is the probability that a pair starts a replacement clutch after the first clutch failed, and NR_R is the average number of recruits from (natural) replacement clutches.

Using NR_{yr} and female survival rate (S_{fem}), the expected lifetime reproductive success (LRS) was calculated as follows:

$$LRS = NR_{yr} + NR_{yr}\cdot(S_{fem} / (1 - S_{fem})).$$

The aim of this exercise is to estimate the fitness associated with a certain reproductive strategy with respect to the timing of reproduction. Therefore, in the calculation it was assumed that a female adopts a certain strategy (= timing of reproduction) for life. Although females that breed at the same date all their lives are probably very rare, females that are early (or late) in one year tend to be also early (or late) in later years (van Noordwijk et al. 1981*b*).

The different fitness components were calculated using data presented in the tables. Selection of values depended on the outcome of the statistical analyses. For example, for P_{suc} in all three calculations, the overall mean of 0.89 was used, as P_{suc} was not related to timing of breeding. For CS, the mean of the four control groups was used for the early group (9.42 eggs). The regression analysis showed that experimental clutches were 0.57 egg smaller, and hence 8.85 eggs was used for the delayed group. CS of the late group was estimated, using the analysis of the natural seasonal trend, by calculating the expected mean CS for birds laying late spontaneously at the same time as the experimental birds (result: 8.19 eggs). For FE, the mean of the four control groups (0.83) was used for the early group and for the experimental group because the seasonal decline in FE was attributed to variation in quality. The FE of the late group was calculated following the same procedure as in calculating their clutch size (result: 0.63). For FR, the mean of the four control groups (0.130) was used for the early group. Because FR was causally related to date, the same value was taken for the delayed group and the late group (0.038), which was the mean of the four experimental groups. The same procedure was followed for the probability that a pair would start a second clutch (early group: 0.12, delayed and late group: 0.0). The number of recruits per second clutch was averaged for each separate

year in which experiments were carried out, and the average of these four years was taken (result: 0.56 recruit per second clutch). Seasonal variation in female survival was attributed to differences in quality and, therefore, the same procedure was followed as with FE (result for control and delayed group: 0.41; for late group: 0.25).

No recruits were produced by nonexperimental replacement clutches ($n = 9$) in the four experimental years; therefore replacement clutches were ignored. A sensitivity analysis, in which the effect of potential values for the number of recruits from replacement clutches was investigated, showed that (nonexperimental) replacement clutches have a negligible effect on LRS due to the low proportion of birds involved.

Using these data, the expected LRS of early females was estimated to be 1.63 recruits, of (spontaneously) late females 0.23 recruits, and of experimentally delayed females 0.42 recruits. The mean laying date interval between the control group and the delayed group (and hence the late group) was 17.2 d. Thus, the natural seasonal trend in LRS had slope of -0.082 recruits per day. (The slope is calculated to make the result independent of the time interval, facilitating comparison with other studies. This should not be taken to imply that we conclude that this relationship is necessarily linear, but is only an estimate of the mean slope over the whole range). The slope between the early group and the delayed group was -0.071. Hence, the natural seasonal trend in LRS is for 87% ($-0.071/-0.082$) to be caused by seasonal changes in the environment, and for the remaining 13% (($-0.082 + 0.071)/-0.082$) to be caused by quality differences between early and late breeders.